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Biography

Alan P. Dawson graduated in Natural Sciences at the University of Cambridge in 1984, and remained in the Department of Biochemistry in Cambridge to study for his PhD with C. J. R. Thorne on the mechanism of glycerophosphate oxidation by mitochondria. Having completed his PhD in 1967, he moved to a lectureship in the School of Biological Sciences in the University of East Anglia, where, like many others who have found the ambience of Norwich and Norfolk far too pleasant to leave, he has remained ever since. During this time his research interests have evolved, via mitochondria and mitochondrial calcium transport, to the mechanism of intracellular calcium homeostasis and the role of inositol phosphates. He is currently a Senior Lecturer in Biochemistry.

Jennifer Rivett received her PhD in Biochemistry from the University of Cambridge in 1980 for studies which were carried out both in Cambridge and at Trinity College Dublin under the supervision of Professor Keith Tipton. She continued to study the enzymology of neurotransmitter metabolism for two years in Buffalo, USA and then moved to Dr Earl Stadtman's laboratory at the National Institutes of Health in Bethesda where she became interested in mechanisms of intracellular protein turnover. Three years later she took up a Medical Research Council Senior Fellowship in the Department of Biochemistry at the University of Leicester, where she has developed her interests in the structure and catalytic properties of high molecular weight intracellular proteinases. She now holds a Lister Institute-Jenner Research Fellowship.

Bjørn Quistorff graduated as MD from The Medical School, University of Copenhagen (1971). He worked at the Department of Biochemistry, University of Copenhagen, with Professor Frank Lundquist and at the Brain Research Laboratory, Lund, Sweden, with Professor B. K. Siesjo. He was appointed Associate Professor at the Department of Biochemistry A, University of Copenhagen (1977). He was a postgraduate fellow at the University of Pennsylvania, Department of Biophysics and Biochemistry (1975–1977), and later a regularly visiting professor, working with Professor Britton Chance. He was appointed adjunct Associate Professor at the University of Pennsylvania (1989). His research has mainly been on metabolic regulation, notably metabolic zonation of the liver. He has

been instrumental in establishing the Magnetic Resonance Center at the Panum Institute, University of Copenhagen. He served as Chairman of The Danish Biochemical Society 1984–1988, and is currently chairman of the Danish National Committee of Biochemistry.

Nicholas P. Kennedy studied Medicine at Dublin University, Trinity College, graduating in 1981. He took an intercalated degree in Biochemistry and obtained his moderatorship in 1978. During his training as a junior hospital doctor, he spent a year working in clinical biochemistry in the Central Pathology Laboratory in St James's Hospital, Dublin, before becoming a Research Fellow in Clinical Medicine. As Research Fellow, he worked in the Medical Research Council of Ireland Alcohol Unit in St James's Hospital, where he was involved in an antenatal screening project to detect maternal alcohol abuse. He undertook training in Gastroenterology in St James's Hospital from 1984 to 1988 and has held the post of Lecturer in Clinical Nutrition in Trinity College, Dublin, since 1984.

His research interests have included peripheral leucocyte eicosanoid metabolism in alcoholic liver disease and in peptic ulcer disease; drug therapy of peptic diseases; the mechanisms of campylobacter infection in gastritis and peptic ulceration; the influence of dietary linoleic acid intake on peptic ulcer disease. He is currently studying the relationship of alcoholism and of alcohol exposure to intestinal mucosal monoamine metabolism.

Keith F. Tipton graduated in Biochemistry at the University of St Andrews in 1962. He worked for his PhD under the supervision of Professor Malcolm Dixon at the University of Cambridge. On being elected to a Fellowship of King's College he remained in Cambridge, first as a University Demonstrator and latterly as a Lecturer in Biochemistry, until 1977. He then moved to Trinity College Dublin where he is Professor of Biochemistry and a Fellow of that College. In research he spends a great deal of time being confused as to whether he is a neurochemist or an enzymologist.

Henry McIlwain's initial work in 1927 was in commercial chemical laboratories and he subsequently held research awards at Newcastle on Tyne and Oxford concerning the organic chemistry of natural products and of free radicals, especially of phenazine derivatives (with G. R.

Clemo and R. Robinson). He joined Medical Research Council units in 1937, first in bacterial chemistry and later in cell metabolism (with P. Fildes and H. A. Krebs). His wartime work was in microbiology, and he subsequently held biochemical posts in Sheffield and with the London County Council Mental Health Services. At the Institute of Psychiatry, London, he was Professor of Biochemistry from 1955 to 1980 and initiated much neurochemical work, especially concerning the metabolism, the excitation and the functioning of neural systems. He was a founder-member of the Society for General Microbiology, of the International Society for Neurochemistry (ISN) and of the Neurochemical Group of the Biochemical Society. He has continued with post-retirement work in neurochemistry, at first in the Division of Biochemistry, UMDS at St Thomas's Hospital Medical School with H. S. Bachelard, supported by the Wellcome Trust; and subsequently in the Department of Pharmacology, Birmingham University Medical School. He is also Historian to the ISN.

Conventions

The abbreviations, conventions and symbols used in these Essays are those specified by the Editorial Board of *The Biochemical Journal* in *Policy of the Journal and Instructions to Authors* (see first issue in latest calendar year). The following abbreviations of compounds, etc., are allowed without definition in the text.

ADP, CDP, GDP, IDP, UDP, XDP, dTDP: 5′-pyrophosphates of adenosine, cytidine, guanosine, inosine, uridine, xanthosine and thymidine.

AMP, etc.: adenosine 5′-phosphate, etc.
ATP, etc.: adenosine 5′-triphosphate, etc.
CM-cellulose: carboxymethylcellulose
CoA and acyl-CoA: coenzyme A and its acyl derivatives
Cyclic AMP, etc.: adenosine 3′,5′-cyclic phosphate, etc.
DEAE-cellulose: diethylaminoethylcellulose
DNA: deoxyribonucleic acid
Dnp-: 2,4-dinitrophenyl-
Dns-: 5-dimethylaminonaphthalene-1-sulphonyl-
EDTA: ethylenediaminetetra-acetate
FAD: flavin adenine dinucleotide
FMN: flavin mononucleotide
GSH, GSSG: glutathione, reduced and oxidized
NAD: nicotinamide adenine dinucleotide
NADP: nicotinamide adenine dinucleotide phosphate
NMN: nicotinamide mononucleotide
P_i,PP_i: orthophosphate, pyrophosphate
RNA: ribonucleic acid (see overleaf)
TEAE-cellulose: triethylammonioethylcellulose
tris: 2-amino-2-hydroxymethylpropane-1,3-diol

The combination NAD^+, NADH is preferred.

The following abbreviations for amino acids and sugars, for use only in presenting sequences and in Tables and Figures, are also allowed without definition.

Amino acids

Ala: alanine
Arg: arginine
Asn: asparagine
Asp: aspartic acid
Asx: aspartic acid or asparagine (undefined)
Cys: cystine or cysteine
Gln: glutamine
Glu: glutamic acid

Glx: glutamic acid or glutamine (undefined)	Ile: isoleucine	Pro: proline
	Leu: leucine	Ser: serine
Gly:glycine	Lys: lysine	Thr: threonine
His: histidine	Met: methionine	Trp: tryptophan
Hyl: hydroxylysine	Orn: ornithine	Tyr: tyrosine
Hyp: hydroxyproline	Phe: phenylalanine	Val: valine

Sugars

Ara: arabinose	Glc*: glucose
dRib: 2-deoxribose	Man: mannose
Fru: fructose	Rib: ribose
Fuc: fucose	Xyl: xylose
Gal: galactose	

* Where unambiguous, G may be used.

Abbreviations for nucleic acids used in these essays are:

mRNA: messenger RNA
nRNA: nuclear RNA
rRNA: ribosomal RNA
tRNA: transfer RNA

Other abbreviations are given on the first page of the text, or at first mention.

References are given in the form used in *The Biochemical Journal*, the last as well as the first page of each article being cited, and, in addition, the title. Titles of journals are abbreviated in accordance with the system employed in the *Chemical Abstracts Service Source Index* (1979) and its Quarterly Supplement (American Chemical Society).

Enzyme Nomenclature

At the first mention of each enzyme in each Essay there is given, whenever possible, the number assigned to it in *Enzyme Nomenclature: Recommendations (1984) of the Nomenclature Committee of the International Union of Biochemistry on the Nomenclature and Classification of Enzyme-catalysed Reactions*, published for the International Union of Biochemistry by Academic Press, New York and London, 1979. Enzyme numbers are given in the form EC 1.2.3.4. The names used by authors of the Essays are not necessarily those recommended by the International Union of Biochemistry.

(a)

(b)

(c)

(d)

(e)

(a) P. N. CAMBELL (Vols 1-20); (b) G. D. GREVILLE (Vols 1-5); (c) F. DICKENS (Vols 6-10) (d) W. N. ALDRIDGE (Vols 11-14); and (e) R. D. MARSHALL (Vols 15-24).

Preface

This is the 25th volume of *Essays in Biochemistry* and thus an appropriate occasion to pay tribute to those who have been responsible for its progress and development since its inception. The originators of the series, Peter Campbell and Guy Greville, were largely responsible for determining the style and format of the books which have persisted to the present time. After Guy Greville's death, Peter continued to devote his energies to editing the series, deciding to retire only after the 20th volume was prepared. During that time he was ably complemented by Frank Dickens, Norman Aldridge and Robin Marshall who, in their turn, acted as co-editors. On Peter's retirement Robin Marshall, as editor, enlisted my help in continuing the series. He decided to retire when volume 24 had been finished.

A glance through the contents lists of earlier volumes will attest the breadth of topics that have been covered in individual essays and the commitment of the editors in obtaining the services of such able authors and in ensuring that their contributions were delivered, more or less, on time.

After 25 years we have decided that some changes are necessary to the format and presentation of the series in order to maintain its value to those involved in teaching, learning and research. This will result in some alterations in the style, organization and content of the next volumes. It is hoped that these developments will enhance the value of the series.

K. F. Tipton

Contents

Regulation of Intracellular Ca^{2+}

ALAN P. DAWSON

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I. Introduction

The key role of intracellular Ca^{2+} in controlling processes such as muscular contraction and secretion has been known for a very long time. The more general role of Ca^{2+} in regulating metabolic processes has emerged more recently with the discovery of calmodulin and other Ca^{2+}-binding regulatory proteins. The study of the mechanisms regulating Ca^{2+} concentrations in cells has evolved in parallel with studies on the processes regulated by Ca^{2+}. The discovery of the relationship between receptor-activated polyphosphoinositide breakdown, inositol 1,4,5-trisphosphate and mobilization of intracellular Ca^{2+} has given great impetus to this area of investigation and has encouraged the study of a very wide variety of cell types.

It is generally agreed that for most animal cell types there is a very steep electrochemical gradient of Ca^{2+} across the plasma membrane. The external free Ca^{2+} concentration is of the order of 1 mM, while the resting intracellular free Ca^{2+} concentration has been found to be in the region of 0·1 μM. Coupled with the concentration gradient, there is also an electrical driving force for Ca^{2+} entry, since the intracellular space is usually found to be at a potential of about −60 mV relative to the outside. Following a Ca^{2+}-mobilizing stimulus, the Ca^{2+} concentration in the

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cytosol is found to rise to about 1 μM in most systems. It is worth noting at this point, however, two rather obvious differences between Ca^{2+} and other intracellular messengers such as cyclic AMP or inositol 1,4,5-trisphosphate. Firstly, Ca^{2+} cannot be synthesized or broken down. The only mechanisms available for causing large-scale changes in Ca^{2+} concentration involve changes in compartmentation, by movements either across the plasma membrane or across intracellular membranes. Secondly, Ca^{2+} ions are "sticky". They bind to a wide variety of ligands such as proteins and phosphate groups. While this is precisely the reason that Ca^{2+} can regulate metabolic processes (coupled with very rapid association and dissociation kinetics), it also means that a very small proportion of intracellular Ca^{2+} is actually the free species, probably $\ll 1\%$.[1] Because of this Ca^{2+} buffering, a change in the cytoplasmic free Ca^{2+} from 0·1 μM to 1 μM actually requires the movement of relatively large quantities of Ca^{2+}.

The regulation of intracellular Ca^{2+} concentration requires interactions between a variety of transport systems in several different membranes. The precise way in which all the systems are integrated to produce a particular end result is in many cases unclear. It is reasonable to assume that the primary source of the concentration gradient between the outside and the inside of the cell must be the Ca^{2+} transport systems of the plasma membrane. However, it does not follow from this that transport mechanisms in the plasma membrane need be primarily responsible for *changes* in intracellular Ca^{2+} concentrations. Historically, one of the best studied (and most specialized) systems is skeletal muscle, where alterations in intracellular Ca^{2+} concentrations arise due to release of Ca^{2+} from, or sequestration of Ca^{2+} into, the intracellular stores of the sarcoplasmic reticulum. In all other cell types which have been examined, at least part of the endoplasmic reticulum appears to be functionally analogous to the sarcoplasmic reticulum, containing a reservoir of potentially mobilizable Ca^{2+}. However, as will be discussed in more detail later, some cell types (e.g. B cells of islets of Langerhans) appear to be totally dependent on entry of extracellular Ca^{2+} for activation, while others (e.g. sea urchin eggs) appear to be wholly independent of extracellular Ca^{2+} under normal circumstances. It seems likely, given the ability of biological systems to make use of any mechanisms which (figuratively) come to hand, that between these extremes there is a more or less continuous spectrum of importance of plasma membrane and endoplasmic reticulum membrane transport systems in causing changes in intracellular Ca^{2+} concentrations.

In the above discussion there is no mention of mitochondrial Ca^{2+} transport. It is now generally agreed that mitochondrial Ca^{2+} transport systems are important, not in determining cytosolic Ca^{2+} concentrations,

but in relaying cytosolic Ca^{2+} concentration changes to the intramitochondrial space.[2] Accordingly, mitochondrial transport systems will be dealt with later from this standpoint.

II. Measurement of Intracellular Ca^{2+}

Fundamental to a study of regulation of intracellular Ca^{2+} concentrations are the methods available for the measurement of values for the free Ca^{2+} concentration. Over the years, these have improved very substantially in terms of general applicability, selectivity and ease of use. Early work was carried out on individual cells, using intracellular microelectrodes[3] or microinjection of Ca^{2+}-sensitive photoproteins such as aequorin or obelin.[4] Originally these methods could only be used on large cells but techniques have improved such that microinjection has now been used on, for example, hepatocytes. Although technically difficult, microinjection gives information about responses of single cells rather than populations. This is important when, for example, Ca^{2+} concentrations oscillate,[5] a response which would probably be averaged out in a large cell population.

One of the earliest measurements of cytosolic free Ca^{2+} on a population of small cells was done using a most ingenious null-point method.[6] The technique was to suspend hepatocytes in media containing various concentrations of free Ca^{2+} and a metallochromic Ca^{2+} indicator (Arsenazo III), then to permeabilize the plasma membranes of the cells with digitonin and measure whether Ca^{2+} was accumulated ($Ca_o > Ca_i$) or released ($Ca_o < Ca_i$) from intracellular stores (Fig. 1) (digitonin is a detergent which binds preferentially to membranes containing steroids, e.g. cholesterol, and therefore makes the plasma membrane permeable without doing too much damage to intracellular membranes). In theory, since the intracellular stores should have been in a steady state with respect to Ca_i, when $Ca_o = Ca_i$ no net movement of Ca^{2+} in or out of the stores should take place on making the plasma membrane permeable to Ca^{2+}. Using this technique, Murphy *et al.*[6] found resting cytosolic Ca^{2+} levels of about 0·1 μM in hepatocytes, rising to about 0·4 μM after adrenaline stimulation.

The null-point method does not, unfortunately, lend itself to continuous measurements. The possibility of this was opened up by the development by Tsien and his colleagues[7,8] of fluorescent Ca^{2+} indicators. The ester forms of the indicators could cross the plasma membrane. Inside the cell, the esters are cleaved by intracellular esterases to leave the free carboxyl groups (Fig. 2), in which form they can no longer pass through the plasma membrane. The entrapped indicator then continuously reports changes

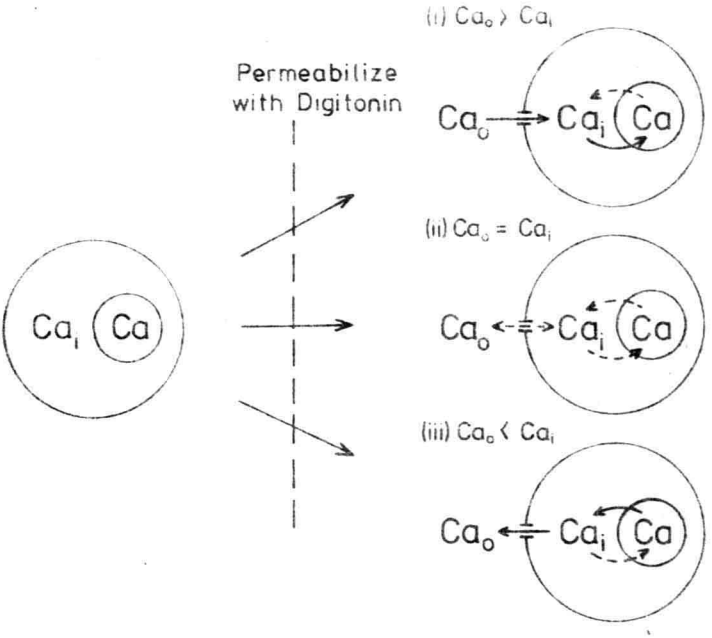
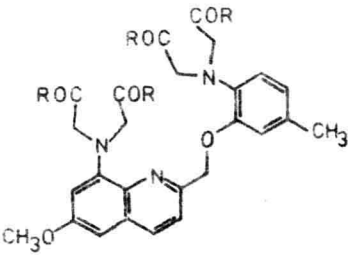


Fig. 1 Null-point technique for determination of intracellular Ca^{2+} concentration. The cartoon on the left represents a cell with an intracellular compartment in a steady state with the cytosolic free Ca^{2+} (Ca_i). On addition of digitonin, to make holes in the plasma membrane, the intracellular stores will be exposed to: (i) an external Ca^{2+} concentration (Ca_o) higher than Ca_i, so that Ca^{2+} will be accumulated by the stores and Ca_o will decrease; (ii) a Ca_o which is the same as Ca_i, in which case the steady state is maintained and there is no net Ca^{2+} movement in either direction; (iii) Ca_o less than Ca_i, in which case Ca^{2+} will come out of the stores and Ca_o will increase. Ca_o was monitored using the metallochromic Ca^{2+} indicator Arsenazo III in the original version of the experiment described in ref. 6.

in intracellular free Ca^{2+}. The earliest compound of this type, Quin 2, has been very extensively used and has produced a great deal of valuable data. However, its fluorescence yield was low, so that rather large concentrations had to be loaded in to give good Ca^{2+} signals. This meant that there was a risk of the measuring system itself perturbing, or at least damping, changes in Ca_i by altering the Ca^{2+} buffering capacity of the cytosol. However, the next generation of indicators of this type, Fura 2 and Indo 1, have much higher fluorescence yields and can therefore be used at much lower intracellular concentrations. It is notable that the use of Fura 2 has enabled small changes in intracellular Ca^{2+} to be measured in systems in which such changes were previously undetected.[9]

(a)



Quin 2 :- $R = -O^-$

Quin 2(AM)$_4$:- $R = -OCH_2OCOCH_3$

(b)

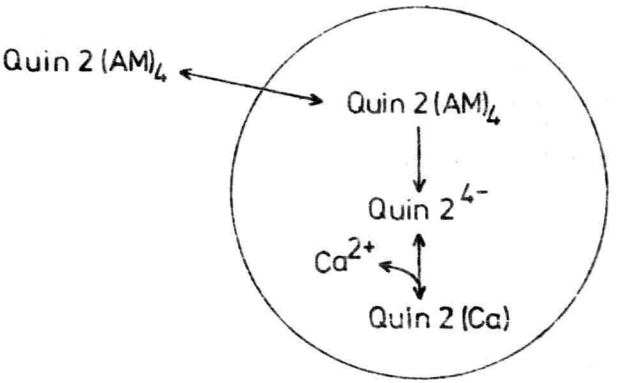


Fig. 2 Structure and use of Quin 2. (a) Shows the structures of Quin 2 and of its tetraacetoxymethyl ester derivative. (b) The tetraacetoxymethyl ester derivative is sufficiently hydrophobic to pass through the plasma membrane. Inside, intracellular esterases hydrolyse off the ester groups, and Quin 2^{4-}, which is too polar to pass through the plasma membrane, is trapped inside, where it accumulates. When the required intracellular concentration of Quin 2^{4-} has been reached, Quin 2 $(AM)_4$ is washed away, leaving a stable Quin 2^{4-} concentration inside to measure intracellular Ca^{2+}.

III. Stimuli Leading to Elevation of Cytosolic Ca^{2+}

Although there is a very large range of hormones, neurotransmitters and other sorts of agonist which influence intracellular Ca^{2+}, they can be conveniently divided into two categories: those working via channels or electrical effects at the plasma membrane and those working via transmembrane chemical signalling to give polyphosphoinositide hydrolysis.

A. ELECTRICAL SIGNALLING

In mammalian skeletal muscle, the stimulus for contraction is the arrival of acetylcholine at the nicotinic acetylcholine receptors on the motor endplate. Although this is clearly a chemical signal, the binding of acetylcholine to its receptor opens Na^+/K^+-conducting channels through the receptor protein, leading directly to depolarization of the membrane.[10] In turn, this leads to propagation of an action potential across the sarcolemma, and release of Ca^{2+} from the sarcoplasmic reticulum. Note, however, that the means of transmission from sarcolemma to sarcoplasmic reticulum is still in doubt (Section IV.A.3), but is probably not chemical. Cardiac and smooth muscle also contract in response to depolarization of the plasma membrane. In cardiac muscle, the regular depolarizations of the sarcolemma lead directly to entry of Ca^{2+} from outside (Sections IV.A.2 and IV.B.3), and contraction is totally dependent on external Ca^{2+}.[11] This is not the case for skeletal muscle. The use of electrical signals in this way is of great biological importance, since depolarization can pass rapidly across a wide area, thereby synchronizing the activity of a large group of muscle cells.

In islets of Langerhans, insulin secretion from B cells can be stimulated by glucose, the addition of glucose leading to membrane depolarization, and the entry of Ca^{2+} from the outside. Groups of B cells within an islet are coupled electrically through gap junctions, so that once again activity is coordinated. Here, however, the scenario is magnificently complicated, since glucose also gives rise to polyphosphoinositide breakdown, which is likely to mobilize intracellular Ca^{2+} as well (see below).

B. TRANSMEMBRANE CHEMICAL SIGNALLING

(1) *Inositol phosphates*

The involvement of phosphoinositides in transmembrane signalling was first indicated by the experiments of Hokin and Hokin,[12] who found that